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Larvicidal activity of *Cyclamen* (Myrsinaceae) extracts against the larvae of West Nile virus vector *Culex pipiens* L. (Diptera: Culicidae)

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ABSTRACT

Objective: To evaluate the larvicidal activity of the tuber extracts isolated from two species of *Cyclamen* (*Cyclamen mirabile* Hildebr. and *Cyclamen alpinum* Dammann ex. Sprenger) against *Culex pipiens* L. (Diptera: Culicidae). **Methods:** Twenty young (first–second) or older (third–fourth) instars of the *Culex pipiens* were exposed to various concentrations (100 to 1 000 ppm) of the extracts. Mortality was recorded after 12–, 24–, 48–, 72– and 96–h of exposure. **Results:** We determined that the young larval stages were more susceptible to the extracts in comparison with the older larval stages. A comparison of LC₅₀ and LC₉₀ values has shown that *Cyclamen mirabile* was more active than *Cyclamen alpinum*. **Conclusions:** This study is the first to report on the larvicidal activity of the extracts of *Cyclamen* species against *Culex pipiens*. More studies are needed to isolate and identify the active components involved, their mode of action, and effects on other mosquito and pest species.

1. Introduction

The genus *Cyclamen* L. (Myrsinaceae) consists of more than 20 species with highest diversity in the Mediterranean area especially Greece and Turkey[1–3]. The genus *Cyclamen* contains 10 species, 4 of which are endemic to Turkey[1]. *Cyclamen* species have a limited dispersal capacity, due to dependence on ants to transport their relatively few, large seeds[4]. The species of this genus grow naturally in dry forest or scrub where they are at least partly shaded from intense sunlight. The flowers are white, pink, purple or carmine with scented or unscented flowers and leaves of many *Cyclamen* species are beautifully marked. Some species belonging to this genus were used for their biological activities in folk medicine. Antiinflammatory and antinociceptive activities of *Cyclamen repandum* Sm. tubers

in rats and mice were shown by Speroni *et al*[5]. Antifungal activity of *Cyclamen mirabile* (*C. mirabile*) Hildebr and *Cyclamen trochopteranthum* O. Schwarz reported by Gundogan *et al*[6].

There are approximately 2 700 species of mosquito in the world; the three most significant genera are the *Anopheles*, *Aedes*, and *Culex*. Many these genera species serve as vectors of important diseases, such as malaria, yellow fever, dengue fever, West Nile virus, St. Louis encephalitis, filariasis, and Japanese encephalitis[7].

The mosquito *Culex pipiens* (*Cx. pipiens*) L. (Diptera: Culicidae) is a common and abundant species in the world. This species serve as bridge vectors of the West Nile virus from birds to humans in the United States[8,9]. The main breeding or larval developmental sites of this species are septic tanks, artificial containers, animal watering basins, water irrigation channels and temporary pools[10]. Control of *Cx. pipiens* in these breeding sites with application of biological and chemical insecticides are main methods to reduce its population[11]. However, this mosquito species has been shown to develop resistance to many insecticides[12].

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The development of new botanical insecticides is important in order to counter the evolution of resistance in *Cx. pipiens* populations. Despite their many biological activities, there is no available data about insecticidal activities of *Cyclamen* genus on this mosquito species.

Therefore, the aim of the present research was to determine the larvicidal activity of extracts of *C. mirabile* (endemic) and *Cyclamen alpinum* (*C. alpinum*) which naturally grow in in Turkey, under laboratory conditions against *Cx. pipiens*.

2. Materials and methods

2.1. Preparation of the extracts

The fresh tubers of both *Cyclamen* species used in the present study were collected from natural habitats in Turkey. Taxonomic identification of *Cyclamen* species was performed by third and fourth authors of this article and voucher species were deposited in the herbarium of Pamukkale University, Denizli, Turkey. The tubers (200 g) were first peeled, cut into 5 mm × 5 mm cubes. These tubers were extracted two times with ethanol at 55 °C. The ethanol extract was then dried under a vacuum in a rotary evaporator and the remaining material was ground into a fine powder. The powder was then lyophilized and stored at 4 °C until usage.

2.2. Mosquito culture

Cx. pipiens used in the assays originated from Arapsuyu, Antalya, and were collected from a pool in August 2011. The larvae were reared at 12:12 light/dark photoperiod, (60±10)% RH, and (26±2) °C in an insectary in the Biology Department, Akdeniz University. The first–second and third–fourth instar larvae were used for bioassays.

2.3. Larvicidal assays

Larvicidal activity of the extracts oils of *C. mirabile* and *C. alpinum* against *Cx. pipiens* was assessed by using the method described by Cetin and Yanikoglu^[13]. For experimental treatment, 0.5 g of each extract was dissolved in 500 mL distilled water. A series of concentrations ranging from 100 to 1 000 ppm of dissolved extract were prepared. The extract–water solution was stirred for 30 s with a glass rod. After approximately 5 min, 20 larvae taken on a strainer with fine mesh were transferred gently to the test medium by tapping. Three replicates of each concentration were run at a time. Mortality was recorded after 12–, 24–, 48– 72– and 96–h of exposure, during which fish food was given to the larvae. All experiments were conducted at (26±2) °C and (60 ±10)% relative humidity with 12:12 D:L photoperiod. Dead larvae were identified when they failed to move after probing with a needle in the siphon or cervical region. Moribund larvae were those incapable of rising to the surface (within a reasonable period of time) or showing the characteristic

diving reaction when the water was disturbed. Larvae were also observed for discoloration, unnatural positions, uncoordination, or rigor.

2.4. Data analysis

When control mortality ranged from 5%–20%, the corrected mortality was calculated using Abbott's formula^[14] and analyzed using Statistical Analysis System ANOVA^[15]. Means were compared with Duncan's multiple range tests. The data were obtained were subjected to probit analysis in order to estimate the LC₅₀ and LC₉₀ values^[16].

3. Results

Toxicities of oils from *C. mirabile* and *C. alpinum* to young (first and second) and older (third and fourth) instar *Cx. pipiens* larvae were noted, and the LC₅₀, LC₉₀, and 95% confidence limits for 72 and 96 h were calculated. The data are presented in Table 1, 3. It was determined that the young larval stages were more susceptible to both extracts in comparison with the older larval stages. *C. mirabile* extract was more toxic than *C. alpinum* extract on young and older larval instars (Table 1). The mean mortality was 3.3% in the control groups for young (first and second) instars and 2.6% for older (third and fourth) instars (Table 2, 3).

The lowest concentration of *C. alpinum* extract (100 ppm) only achieved 16.6% mortality of mosquitoes at 72 h post exposure but at 96 h mortality significantly increased to 40.0%. The mortality was not generally concentration and time dependent in the young instar larvae (Table 2). *C. alpinum* extract caused ≥80.0% mortality on first and second larval stages of the species at 72 h at the doses of 750 and 1 000 ppm (Table 2).

After 72 h of exposure, over 90% mortality in first and second instars was observed from treatment with *C. mirabile* extract at concentrations greater than 400 ppm. After 96 h of exposure, over 53% mortality in both young and the older instars was obtained at all concentrations of *C. mirabile* extract tested with the exception of 100 ppm in the older instar larvae (Table 3).

4. Discussion

The insecticidal activity of plant based products (essential oils and extracts) against different mosquito species has been evaluated by many authors^[17–19]. The larvicidal bioassays performed by Kumar *et al*^[20] on early fourth instar larvae of *Anopheles stephensi* Liston and *Aedes aegypti* (*Ae. aegypti*) L. with hexane and petroleum ether extracts of *Citrus limetta* peels. They reported that all treatments resulted in complete mortality and LC₅₀ values of 132.45 and 96.15 ppm against *Anopheles stephensi* and *Ae. aegypti*, respectively; while the petroleum ether extracts from the *Citrus limetta* peels showed LC₅₀ values of 244.59 and 145.50 ppm. The larvicidal

activities of methanolic fractions from *Adhatoda vasica* leaf extracts were investigated against the *Culex quinquefasciatus* (*Cx. quinquefasciatus*) Say and *Ae. aegypti* by Thanigaivel[21]. These authors showed that all the tested fractions proved to have strong larvicidal activity against *Cx. quinquefasciatus* and *Ae. aegypti*. Some Labiatae (Lamiaceae) plant ethanol extracts have a remarkable toxicity against the fourth instars larvae of *Cx. pipiens*. LC₅₀ values of *Teucrium divaricatum* Sieber, *Mentha longifolia* (L.) Huds., *Melissa officinalis* L., *Salvia sclarea* L. and *Mentha pulegium* L. are 18.6, 26.8, 39.1, 62.7 and 81.0 ppm, respectively[22].

The methanol leaf extracts of some *Vitex* species were used for larvicidal assay with LC₅₀ values; *Vitex negundo*, 212.5 ppm, *Vitex trifolia* 41.4 ppm, *Vitex peduncularis* 76.2 ppm,

and *Vitex altissima* 128.0 ppm, against the early fourth-instar larvae of *Cx. quinquefasciatus*[23]. The LC₅₀ values of methanol, chloroform, benzene, ethyl acetate, and hexane extracts of *Eclipta alba* (L.) Hassk (Asteraceae) against early third instar larvae of *Ae. aegypti* were 127.6, 146.2, 151.3, 154.8, and 165.1 ppm respectively[24]. Larvicidal efficacy of *Cassia fistula* Linn. leaf extract against *Culex tritaeniorhynchus* Giles and *Anopheles subpictus* Grassi was evaluated[25]. The methanolic extract of *Cassia fistula* showed highest larvicidal activity against both mosquito species.

Until now, biological activities of extracts isolated from *Cyclamen* species have been studied by different authors in the world. High antioxidant activities of petroleum

Table 1

LC₅₀ and LC₉₀ (72 and 96 h) values (ppm) of the extracts of *C. alpinum* and *C. mirabile* against *Cx. pipiens*.

Plant species	Time (h)	LC ₅₀	% 95 CL (LCL–UCL)	LC ₉₀	Limits
First and second instars	<i>C. alpinum</i> 72	289.7	201.9–371.0	846.5	624.9–1504.1
	<i>C. alpinum</i> 96	143.8	62.1–210.2	498.8	346.7–1056.3
	<i>C. mirabile</i> 72	165.3	57.1–250.7	493.8	326.0–1414.7
	<i>C. mirabile</i> 96	86.2	25.5–132.0	253.7	176.8–453.6
Third and fourth instars	<i>C. alpinum</i> 72	503.6	397.3–621.5	1 300.7	957.4–2476.7
	<i>C. alpinum</i> 96	262.5	224.8–298.8	924.1	779.3–1156.7
	<i>C. mirabile</i> 72	294.8	214.2–352.0	470.1	390.2–718.4
	<i>C. mirabile</i> 96	161.3	87.1–221.7	422.3	308.3–761.9

95% CL confidence limit at 95%, LCL lower confidence limit, UCL upper confidence limit.

Table 2

Larvicidal activity the extracts of *C. alpinum* against *Cx. pipiens* (% Mortality ± SE).

Exposure times (h)		Test concentrations (ppm)							
		0	100	200	300	400	500	750	1 000
First and second instars	12	0.0±0.0	0.0±0.0	26.6±6.6	30.0±5.7	36.6±8.8	40.0±10.0	46.6±6.6	60.0±5.7
	24	3.3±3.3	0.0±0.0	30.0±5.7	30.0±5.7	46.6±3.3	43.3±8.8	46.6±6.6	65.0±2.8
	48	3.3±3.3	16.6±3.3	43.3±3.3	46.6±6.6	50.0±5.7	60.0±5.7	70.0±5.7	76.6±6.6
	72	3.3±3.3	16.6±3.3	40.0±5.7	46.6±6.6	53.3±3.3	83.3±6.6	86.6±3.3	96.6±3.3
	96	3.3±3.3	43.3±3.3	66.6±8.8	73.3±6.7	73.3±6.6	93.3±6.6	100.0±0.0	100.0±0.0
Third and fourth instars	12	2.6±2.6	0.0±0.0	0.0±0.0	6.6±3.3	3.3±3.3	6.6±6.6	15.0±8.6	13.3±6.6
	24	2.6±2.6	0.0±0.0	0.0±0.0	6.6±3.3	13.3±8.8	20.0±5.7	15.0±8.6	30.0±0.0
	48	2.6±2.6	3.3±3.3	0.0±0.0	23.3±3.3	30.0±5.7	23.3±3.3	30.0±5.7	53.3±3.3
	72	2.6±2.6	3.3±3.3	10.0±5.7	33.3±3.3	56.6±5.7	60.0±0.0	80.0±3.3	86.6±2.6
	96	2.6±2.6	13.3±3.3	40.0±0.0	53.3±3.3	73.3±8.8	86.6±3.3	90.0±5.7	100.0±0.0

Control: Distilled water.

Table 3

Larvicidal activity the extracts of *C. mirabile* against *Cx. pipiens* (% Mortality ± SE).

Exposure times (h)		Test concentrations (ppm)							
		0	100	200	300	400	500	750	1 000
First and second instars	12	0.0±0.0	0.0±0.0	20.0±4.0	24.0±6.9	40.0±5.7	40.0±5.7	65.0±2.8	95.0±2.8
	24	3.3±3.3	5.3±0.6	20.0±4.0	24.0±6.9	60.0±5.7	56.6±8.8	70.0±5.7	95.0±2.8
	48	3.3±3.3	16.0±4.0	40.0±8.0	43.3±5.7	73.3±8.8	73.3±8.8	100.0±0.0	100.0±0.0
	72	3.3±3.3	44.0±8.1	52.0±8.0	56.6±6.6	90.0±5.7	93.3±3.3	100.0±0.0	100.0±0.0
	96	3.3±3.3	66.6±3.3	73.3±8.8	90.0±5.78	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0
Third and fourth instars	12	2.6±2.6	0.0±0.0	0.0±0.0	3.3±3.3	16.6±6.6	18.6±1.3	45.0±2.8	70.0±2.8
	24	2.6±2.6	8.0±4.0	10.6±1.3	10.6±1.3	20.0±5.7	26.6±3.3	65.0±2.8	90.0±2.8
	48	2.6±2.6	8.0±4.0	16.6±6.6	28.0±10.6	46.6±8.8	73.3±6.6	80.0±0.0	100.0±0.0
	72	2.6±2.6	12.0±6.9	23.3±3.3	60.0±5.7	66.6±8.8	100.0±0.0	100.0±0.0	100.0±0.0
	96	2.6±2.6	40.0±4.0	53.3±3.3	70.0±5.7	86.6±3.3	100.0±0.0	100.0±0.0	100.0±0.0

Control: Distilled water.

ether, acetone, methanol and water extracts of *C. mirabile* leaves and tubers reported by Sarikurkcu[26]. The anti-biofilm activity (Inhibition concentration₅₀ \leq 32 μ g/mL) of *Cyclamen hederifolium* was demonstrated by Quave et al[27]. But, there is no available data about insecticidal activities of *Cyclamen* genus on mosquitoes. This study is the first to report on the larvicidal activity of the extracts of *Cyclamen* species against *Cx. pipiens*. More studies are needed to isolate and identify the active components involved, their mode of action, and effects on other mosquito and pest species.

Conflict of interest statement

We declare that we have no conflict of interest.

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